

Page 33, line 8, cancel "succinimide" and insert  
--succinamide--.

In the Claims:

Please cancel the claims and insert the following new claims:

Claim <sup>28</sup>35. A method for measuring the concentration of free thyroxine or triiodothyronine ligands in biological fluids in the presence of bound ligand and endogenous binding proteins, including albumin, without disturbing the equilibrium between the free ligand and the protein bound ligand, comprised of the following steps:

(a) incubating a sample of the biological fluid with (i) a ligand analog tracer which, due to its chemical structure, does not bind to some of the endogenous binding proteins, (ii) a specific ligand binder at a concentration which does not significantly strip bound ligand from said endogenous proteins and having an affinity constant from about  $0.246 \times 10^5$  up to about  $5 \times 10^5$  l/mol and, (iii) at least one specific chemical inhibitor reagent that inhibits the binding of the ligand analog tracer to other endogenous binding proteins, said specific chemical inhibitor reagent being present in a concentration sufficient to displace the ligand analog tracer from at least one other endogenous binding protein without

displacing the native ligand from said endogenous binding proteins;

(b) separating the ligand analog tracer bound to the specific binder from unbound tracer; and

(c) determining the concentration of free ligand in said biological fluid.

Claim 36. A method for measuring the concentration of free thyroxine or triiodothyronine free ligands in biological fluids in the presence of bound ligand and endogenous binding proteins, including albumin, without disturbing the equilibrium between the free ligand and the protein bound ligand, comprised of the following steps:

(a) incubating a sample of biological fluid with (i) a ligand analog tracer which, due to its chemical structure, does not bind to some of the endogenous binding proteins, (ii) a specific ligand binder at a concentration which does not significantly strip bound ligand from said endogenous proteins and having an affinity constant from about  $0.246 \times 10^5$  up to about  $5 \times 10^5$  l/mol and, (iii) specific chemical inhibitor reagents that alone or in combination inhibit the binding of the ligand analog tracer to other endogenous binding proteins, said specific chemical inhibitor reagents being present in a concentration sufficient to displace the ligand analog tracer from at least one other endogenous binding protein without

displacing the native ligand from said endogenous binding proteins;

(b) separating the ligand analog tracer bound to the specific binder from unbound tracer; and

(c) comparing the bound fraction in said sample to the bound fraction of a given set of known free ligand calibrators to determine the concentration of free ligand in said biological fluid.

Claim <sup>30</sup>37. The method of claim <sup>28</sup>35 wherein the chemical inhibitor agent is 2,4-dinitrophenol at a concentration of 5-10 mmol/l.

Claim <sup>31</sup>38. The method of claim <sup>28</sup>35 wherein the chemical inhibitor agent is sodium salicylate at a concentration of 40-125 mmol/l.

Claim <sup>32</sup>39. The method of claim <sup>28</sup>35 wherein the chemical inhibitor reagent is sulfobromophthalein at a concentration of  $0.8 \times 10^{-5}$  M to  $1.6 \times 10^{-5}$  M.

Claim <sup>33</sup>40. The method of claim <sup>28</sup>35 wherein the chemical inhibitor reagent is oleic acid at a concentration of 0.4-0.8 mmol/l.

Claim <sup>34</sup>~~41~~. The method according to claim <sup>28</sup>35 or <sup>29</sup>36 wherein the specific ligand binder is an antibody to said free ligand.

Claim <sup>35</sup>~~42~~. The method according to claim <sup>28</sup>35 or <sup>29</sup>36 wherein the specific ligand binder is immobilized on a solid substrate.

Claim <sup>36</sup>~~43~~. A method according to claim <sup>35</sup>42 wherein the solid substrate is polypropylene.

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CONT.  
Claim <sup>37</sup>~~44~~. The method according to claims <sup>28</sup>35 to <sup>29</sup>36 wherein the ligand analog tracer is labelled with at least one radioactive atom, an enzyme, fluorophor, light chromophore or chemiluminescent group.

Claim <sup>38</sup>~~45~~. The method according to claim <sup>37</sup>44 wherein the ligand analog tracer is N-<sup>125</sup>I-L-triiodothyronine succinimide or N-<sup>125</sup>I-L-thyroxine succinimide.

Claim <sup>39</sup>~~46~~. The method according to claims <sup>28</sup>35 or <sup>29</sup>36 when carried out at about 37°C and at about pH 7.4.

Claim <sup>40</sup>~~48~~. The method according to claim <sup>29</sup>36 wherein said free ligand calibrators have been prepared by adding different amounts of the ligand to ligand-free human serum, calibrating by equilibrium dialysis and assigning free ligand values.

Respectfully submitted,

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